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DEVELOPMENT OF ASYMMETRY IN THE NEUROMUSCULAR SYSTEM OF LOBSTER CLAWS

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ABSTRACT

The paired claws of the lobster *Homarus americanus* which are symmetrical in form and function in the larval and early juvenile stages gradually transform into a slender, fast-acting cutter claw and a stout, slow-acting crusher claw during later juvenile and adult stages. Correspondingly changes occur in the neuromuscular system of the claws. The paired claw-closer muscles are initially symmetrical in their fiber composition and consist of a band of fast fibers sandwiched on either side by slow fibers. During development one of the muscles transforms into a cutter with a majority of fast fibers and a small ventral band of slow fibers and the other muscle transforms into a crusher with only slow fibers. The firing pattern of the juvenile fast closer excitor motoneuron consisting of high frequency, long duration bursts, is essentially retained in the adult crusher but changed in the adult cutter to low frequency, short duration bursts. In the paired juvenile closer muscles almost all fibers receive mixed innervation from both fast and slow axons whereas in the adult cutter muscle innervation by the fast axon predominates while in the crusher both are equitably distributed. The development of asymmetry in the closer muscle is regulated by impulse-mediated muscle tension though how the neural asymmetry arises is unknown, but amenable to experimentation.

INTRODUCTION

The body plan of many higher animals from annelids to vertebrates is characterized by symmetry of the left and right sides. Within this bilaterally symmetrical organization, however, asymmetries arise manifested most dramatically by cerebral lateralization in humans (Corballis and Morgan, 1978), vocalization in songbirds (Nottebohm, 1977), and cheliped laterality in crustaceans (Przibram, 1901). Despite the tremendous interest throughout the ages in human laterality we still do not understand how it or any of the other biological asymmetries in the animal world is acquired. One of the more compelling hypotheses put forward by Corballis and Morgan (1978) attributes cerebral lateralization to a left-right maturational gradient. According to this scheme both sides are equipotent initially, but mature at different rates subsequently, with the left leading and at the same time suppressing the right; thereby resulting in the left cerebral hemisphere being dominant for speech and verbal processes while the right deals with non-verbal input. This also explains why when the left side is damaged or lesioned the right side is more disposed to take over its function than vice-versa. Among certain songbirds sectioning of the left hypoglossal nerve, but not the right, severely disrupts the singing pattern and demonstrates the lateralization of singing which includes not only the efferent pathway but the associated nuclei in the brain (Nottebohm, 1977). However, the right side can take over control of singing if the

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left hypoglossus is sectioned before the onset of spring song suggesting that both sides have the capacity for singing but its expression is normally limited to the left side. These examples of asymmetry point to a bias during development which may be profitably studied among crustaceans such as the lobster *Homarus americanus.*

The paired chelipeds or claws of the adult lobster are asymmetric in form and function consisting of a greatly enlarged, slow acting, and powerful crusher claw either on the left or right side, and a more slender, fast-acting cutter claw on the opposite side (Herrick, 1895). Yet in the larval and early juvenile stages the paired claws are symmetrical and equipotent. The neuromuscular system within these claws has received considerable attention because of their relative simplicity: there are only two muscles each innervated by few motoneurons (Wiersma, 1955). It is the development of asymmetry in the neuromuscular system of the paired claws that is reviewed here as part of an ongoing study to understand the biological basis of asymmetry.

Development of Asymmetric Claws

The natural history of the east coast lobster *Homarus americanus* is given in narrative detail in the two voluminous works of Herrick (1895, 1911). The adult female bears eggs every second year. Following a molt usually in July she copulates with a male and subsequently extrudes fertilized eggs. These eggs are carried attached to the swimmerets until the following spring when they hatch as myesid larvae. All three larval stages are planktonic, swimming by means of fan-shaped expodites on their thoracic appendages (Neal *et al.,* 1976). In all three larval stages the paired claws are symmetrical in form (Fig. 1) and slightly larger than the walking legs. They have a few conspicuous sensory bristles but do not have any teeth on their biting surfaces which is characteristic of the adults. At the molt to the 4th stage, which is the first juvenile stage, the animal transforms to a diminutive lobster in that it loses the expodites, and now swims by means of its swimmerets located on the abdomen. At

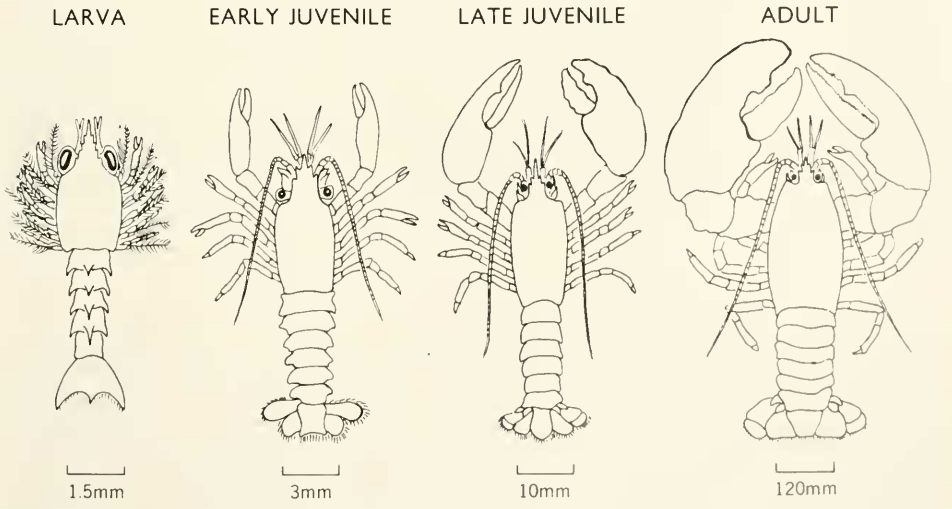


FIGURE 1. Development of the paired claws of the lobster beginning from a symmetrical condition in the larval (1st) and an early juvenile (4th) stage to an increasingly asymmetrical condition in a late juvenile (12th) and an adult stage. Magnification: larva 13×; early juvenile 7×; late juvenile 2×; adult 0.2×.

the same time the claws elongate disproportionately compared to the walking legs and are held extended in front of the animal. The biting surfaces in particular are covered with sensory hair and show the first signs of dentition, usually a single central tooth on the pollex. The paired claws in the 4th, 5th, and 6th stage are symmetrical in form but begin to differentiate in the succeeding stages with the putative cutter claw remaining long and slender and the putative crusher becoming short and stout. The other characteristic change concerns the central tooth on the pollex which remains sharp and narrow (incisor-like) in the cutter while becoming rounded and broad (molar-like) in the crusher. The differentiation in external morphology continues until in the adult the paired claws consist of a distinct cutter and crusher claw. Indeed the claws appear to continue elaborating their distinct external form as the asymmetry becomes even more striking in large adults. It is known that the claws grow in a positive allometric fashion compared to the rest of the body (Lang *et al.*, 1977c) throughout the life of the lobster.

Less is known about the development of functional differentiation between the paired claws. Casual observation in the larval stage show the claws to be used in grasping food. This is supplemented in the early juvenile stages by "meral display" in which the paired claws are held extended and open in a threatening or defensive posture. In these early stages the claw closes at a variety of speeds ranging from approximately 50 to 400 ms (Hill and Govind, 1984). Both claws display this range of closing speeds. It is only in late juveniles and early adults that a clear distinction in closing speeds occurs between the asymmetric claws (Govind and Lang, 1974, 1979). Now the cutter claw displays both fast and slow closing speeds while the crusher closes only slowly. In isolated claws stimulation of the fast closer excitor (FCE) axon with 2 impulses 6.5 ms apart closes the cutter claw in 20 ms while in the crusher claw the homologous axon required 8 impulses, 5 ms apart to cause closing in 90 ms. The closing behavior fatigues more readily and at a lower frequency of stimulation of the FCE in the cutter than in the crusher claw. An essentially similar differentiation in closing behavior was seen between the crusher and cutter claws with stimulation of the slow excitor (SCE) axon. Thus tonic contractions were observed at a lower stimulus frequency and they fatigued more rapidly in the cutter than in the crusher claw. Overall closing of the crusher claw is much slower and more powerful than in its cutter counterpart with stimulation of the homologous motoneurons. The difference in closing behaviors between the paired asymmetric claws is seen in all sizes of adult lobsters including some very large animals, thus suggesting that the functional differentiation is maintained throughout the life span of the lobster. How this functional dissimilarity develops will be traced by examining the muscular and neural substrates governing claw behavior.

Development of Muscle Asymmetry

The lobster claw represents a relatively simple motor system having only two antagonistic muscles (Fig. 2). Both muscles are bipinnate in form and run the length of the propus. The opener muscle is relatively small occupying 10% of the claw muscle mass while the massive closer makes up the other 90%. The opener muscle is situated distally and its contraction opens the dactyl: the closer muscle closes the dactyl on the pollex. Most of the work on the development of asymmetry has been done on the closer muscle because it is responsible for the striking differences in closing behavior of the cutter and crusher claws. On the other hand such differences are not obvious in the opening behavior and consequently the opener muscle has received scant attention.

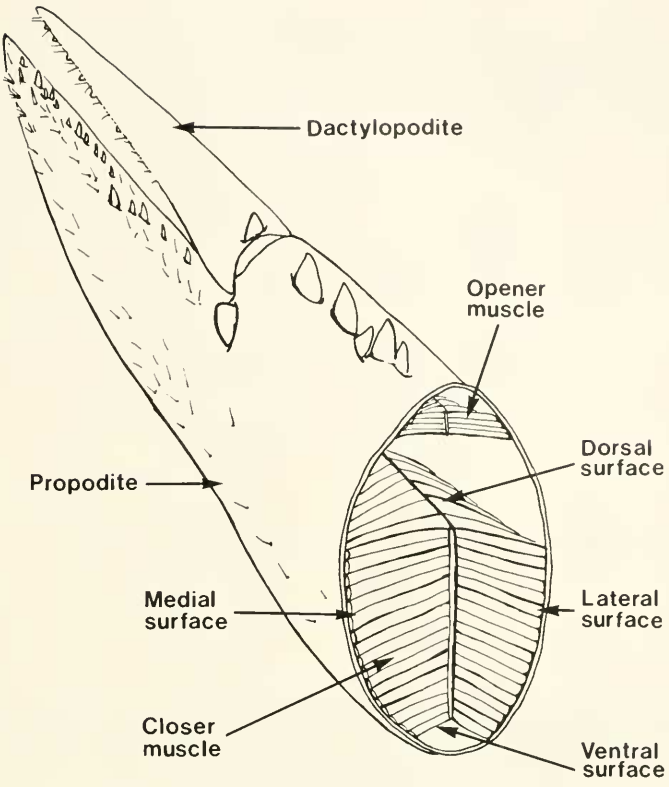


FIGURE 2. Cut-away diagram of an adult cutter claw showing the location and relative size of the antagonistic opener and closer muscles (from Govind and Lang, 1974).

Closer muscle

The development of the closer muscle in the paired claws has been extensively investigated especially with regard to its fiber composition using contractile, structural, histochemical, and biochemical characteristics. The overall picture obtained from all these studies is the symmetry in fiber composition of the paired muscles in the larval and early juvenile stages with the gradual differentiation into a cutter muscle with predominantly fast fibers and some slow fibers and a crusher muscle with all slow fibers.

Structural properties. Unlike vertebrate muscle in which the different fiber types of fast-twitch and slow-twitch have a uniform sarcomere length (SL) of 2–4 μm, crustacean muscle has a wide range of SL from 2–20 μm (Govind and Atwood, 1982). Early studies by Atwood and his collaborators (reviewed by Atwood, 1967, 1973) established that short SL (2–4 μm) fibers are fast-contracting while long SL (>6 μm) fibers are slow-contracting. Using this scheme the fiber composition of the paired closer muscle was determined during development (Jahromi and Atwood, 1971; Goudey and Lang, 1974; Lang *et al.*, 1977a, b, c; Govind and Lang, 1978; Costello and Lang, 1979) and is summarized in Table I and Figure 3. The grouping of sarcomeres into the three categories of short <4 μm, intermediate 4–6 μm, and long >6 μm, in Table I was based on the prevailing dogma that these represented respectively fast,

TABLE I

Fiber composition based on sarcomere length of the paired claw closer muscles during development of the lobster

Stage	No. of animals	Length of animal (mm)	% of fiber types based on sarcomere length (μm)*					
			Claw I (cutter)			Claw II (crusher)		
			Fast <4	Inter-mediate 4–6	Slow >6	Fast <4	Inter-mediate 4–6	Slow >6
Larval								
1	3	7.5	39	58	3	29	68	3
2	3	8.5	43	35	2	40	56	4
3	5	10	54	45	21	25	54	21
Early Juvenile								
4	7	12	36	5	59	26	3	71
5	5	14	50	1	49	27	1	72
Late Juvenile								
6	4	16	56	0	44	21	1	78
11	2	32	73	1	26	23	1	76
13	1	39	64	0	36	0	0	100
15	1	55	82	0	18	4	0	96
Adult								
?	1	250	63	0	37	0	4	96

* The number of fibers sampled from each closer muscle was 30 for the larval stages, 60 for the juvenile 4th stage, and 90 for the remainder (from Lang *et al.*, 1977a, b; and Govind and Lang, 1978).

intermediate, and slow fiber types (Atwood, 1967, 1973). According to the scheme the first two larval stages have predominantly short and intermediate SL fibers. There is a substantial increase in the number of long SL fibers at the 3rd (larval) stage, and again at the 4th and 5th (early juvenile) stages. These increases occur at the expense of the intermediate SL fibers so that by the 5th stage there are few fibers of intermediate SL. These data show a lengthening of the SL from intermediate to long during development of the larval and early juvenile stages. Such lengthening of the sarcomeres appears to be a normal process of crustacean muscle development (Bittner, 1968; Govind *et al.*, 1974; Bittner and Traut, 1978). Over and above this growth-related process, the closer muscle shows two distinct populations of relatively short and long SL fibers in the larval and early juvenile stages (Table I). This dichotomy is graphically represented in the histograms of SL (Fig. 3) where for the larval stage, the fibers separate into the categories of <4 μm and >6 μm. Development of the closer muscle up to the early (4th and 5th) juvenile stages shows a distinct separation of short and long SL fibers. This distribution is seen in both of the paired muscles.

The asymmetry between the paired muscles occurs in the succeeding juvenile stages. Beginning with the 6th stage the population of short SL (<4 μm) increases in one of the paired claws while the population of long SL (>6 μm) fibers increases in the other claw. As a result of these changes in SL, the cutter muscle ends up with predominantly (60–80%) short SL fibers and the remainder long SL fibers while the crusher muscle ends up with all long SL fibers. The asymmetry of the paired closer muscles characteristic of the adult is usually established by the 13th stage which represents the first year of juvenile development. It takes between 5–7 years for lobsters to mature into adults (Hughes *et al.*, 1972).

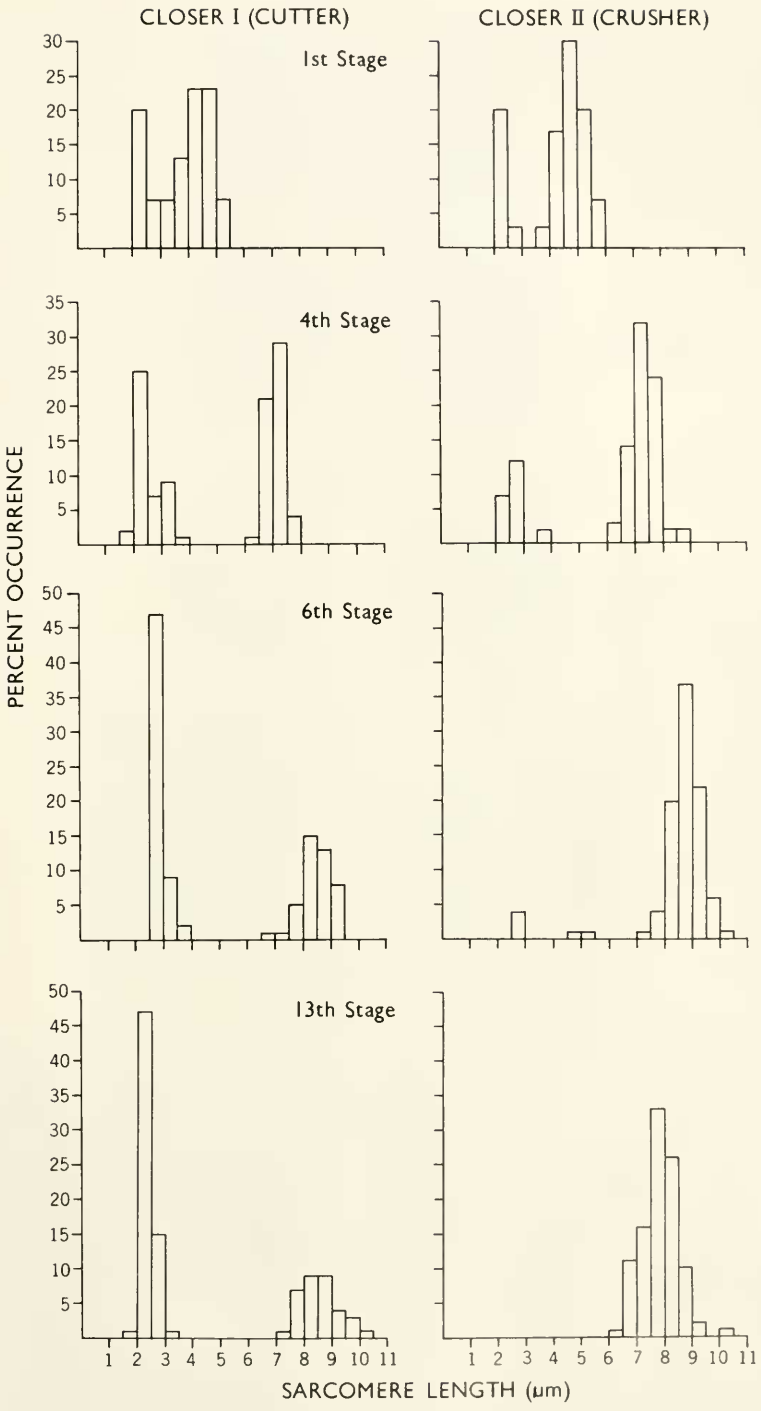
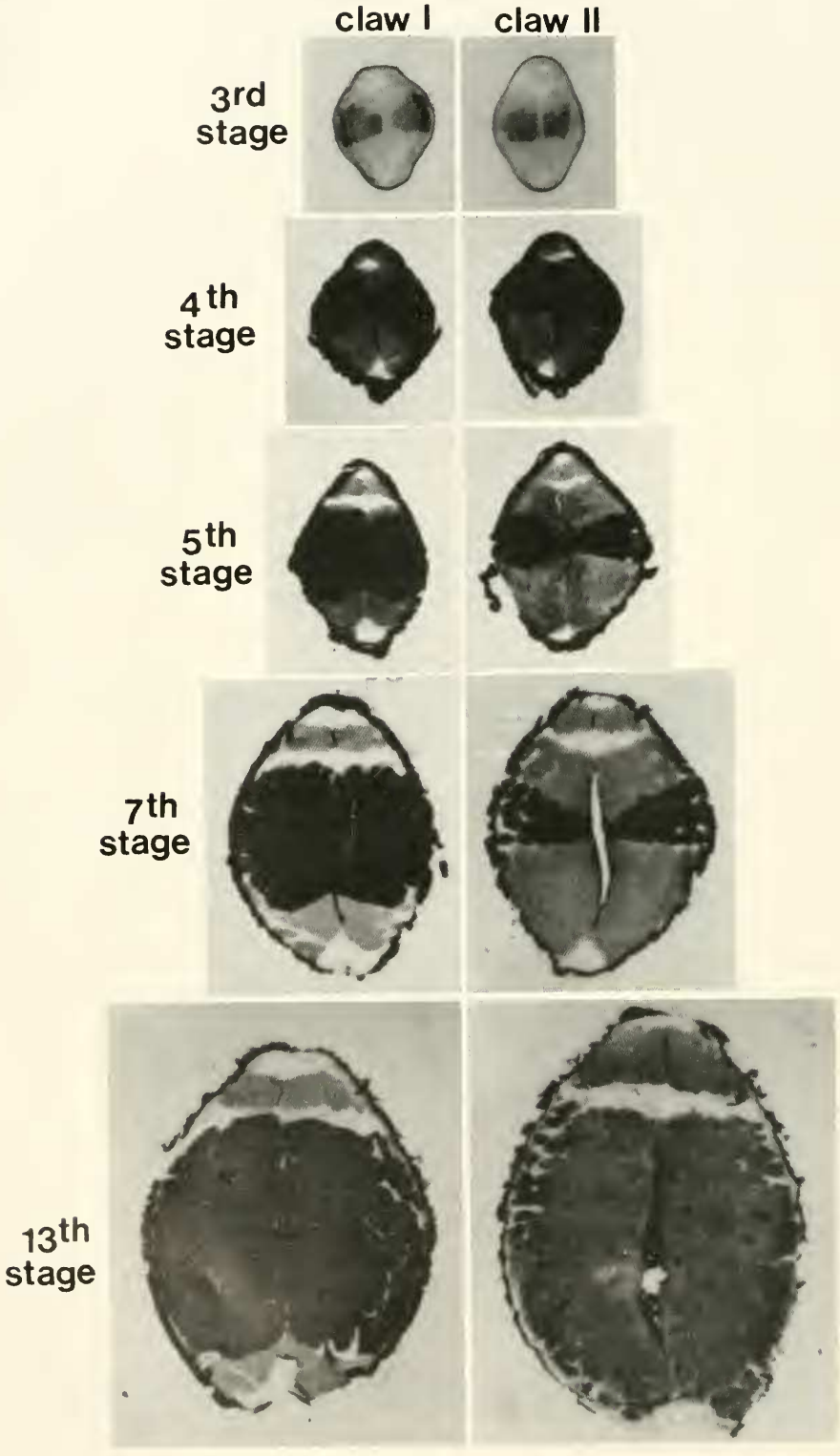


FIGURE 3. Histograms of percent occurrence of muscle fiber types based on sarcomere length in the paired claw closer muscles during development as represented by a larval (1st) and several juvenile (4th, 6th, 13th) stages. Number of fibers sampled for each claw is 30 for the 1st stage, 60 for the 4th stage, and 90 for the remaining stages (from Lang *et al.*, 1977a, b, Govind and Lang, 1978).


claw I
claw II
3rd stage
4th stage
5th stage
7th stage
13th stage

The transformation of the paired claw closer muscles from the symmetrical to the asymmetrical condition involves the acquisition of short SL, presumably fast, fibers in the putative cutter claw and of long SL, presumably slow, fibers in the putative crusher claw. Since no evidence for degenerating fibers has been found, the changeover to short and long SL fibers in the appropriate claws must be due to the transformation of existing fibers. The transformation from short to long SL fibers in the development of the crusher claw may be explained by the lengthening of sarcomeres: a process which has been amply demonstrated among crustacean muscle fibers. However, the transformation of long to short SL fibers during development of the cutter claw is not as easily explained. They could arise by longitudinal splitting of existing short SL fibers; a mechanism which has been suggested to account for growth of a lobster leg muscle (El-Haj *et al.*, 1984), or the short SL fibers could arise by transverse splitting of sarcomeres at their H-bands as has been shown to occur in an adult crab muscle (Jahromi and Charlton, 1978).

Histochemical properties. Among vertebrates determination of muscle fiber types using enzyme histochemistry for the detection of myofibrillar adenosinetriphosphatase (ATPase) activity is well established (review by Burke, 1981). Such histochemical techniques have only more recently been applied to crustacean muscle (Ogonowski and Lang, 1979) where fast muscle stains more intensely than slow since the specific activity of myofibrillar ATPase of crustacean fast muscle is two to three times greater than that of slow muscle (Hajek *et al.*, 1973; Lehman and Szent-Györgyi, 1975).

The differentiation of fiber types in the paired closer muscles was followed in a larval and several juvenile stages (Fig. 4) (Ogonowski *et al.*, 1980). In the 3rd larval stage the paired muscles were symmetrical in their fiber composition consisting of a central band of dark-staining fast fibers sandwiched by light-staining, slow fibers on the dorsal and ventral surfaces. Histochemistry of the 1st and 2nd stage larval claws revealed little if any staining for ATPase in the muscles suggesting that the fibers had little (if any) of this enzyme in these early developmental stages. The symmetry in fiber composition between the paired muscles is also seen in the juvenile 4th stage, though occasionally slight asymmetries in the width of the central dark-staining band are present (Fig. 4). In the 5th stage one of the claws has the central fast band consistently broader than that of its counterpart claw. This is the putative cutter claw where the fast fibers continue to be elaborated over most of the closer muscle except for a narrow ventral strip in the succeeding juvenile stages until the process is completed by the 9th–10th stage. The other claw differentiates into the crusher by the expansion of slow fibers dorsally and ventrally and the diminution of the central fast band until about the 13th stage when the muscle is composed of all slow fibers. Thus at the end of the first year of development the paired closer muscles are differentiated into their asymmetric condition. The cutter muscle has predominantly fast fibers and a small ventro-lateral band of slow while the crusher muscle has all slow fibers. Among the slow fibers in both claws there is a small sub-population located distally which are slower than the remaining majority (Kent and Govind, 1981.)

The development of asymmetry in the paired closer muscles from a symmetric condition occurs by the transformation of slow to fast fibers in the putative cutter claw and of fast to slow in the putative crusher claw. This is suggested by the observation of fibers with an intermediate staining intensity than that characteristic of fast and

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FIGURE 4. Representative cross-sections stained for myofibrillar ATPase activity showing distribution of fast (dark-staining) and slow (light-staining) fibers during development of the paired closer muscles in a larval (3rd) and several juvenile (4th, 5th, 7th, 13th) stage lobsters. The small dorsally situated opener muscle retains its slow (light-staining) character throughout development (from Ogonowski *et al.*, 1980).

slow fibers. Thus in the putative cutter muscle these intermediate type fibers were found in the dorsal region which is destined to become fast while in the putative crusher muscle they were found in the central region which is destined to become slow. (Fig. 4). Such changeovers in the enzymatic profile of fibers have been shown to occur between the fast-twitch and slow-twitch fibers in vertebrate muscle and to be under the direction of the innervating motoneurons (reviewed by Guth, 1968; Gutmann, 1976; Harris, 1974; Jolesz and Sreter, 1981).

Biochemical properties. The protein composition of the closer muscle in juvenile and adult lobsters has been examined using gel electrophoresis (Costello and Govind, 1984). Adult fast and slow muscle have several proteins in common and these are listed along with their molecular weights as follows: (Fig. 5) myosin heavy chain (HC, 154K), two myosin light chains (LC1, 20K in doublet form and LC2, 16K), actin (A, 41K), tropomyosin (TM, 34K), and a protein tentatively identified as α-actinin at 92K. Another major protein tentatively identified as paramyosin (P) differs in molecular weight between fast and slow muscle at 99K and 96K respectively. Apart from these common proteins, adult fast and slow muscle have proteins unique to

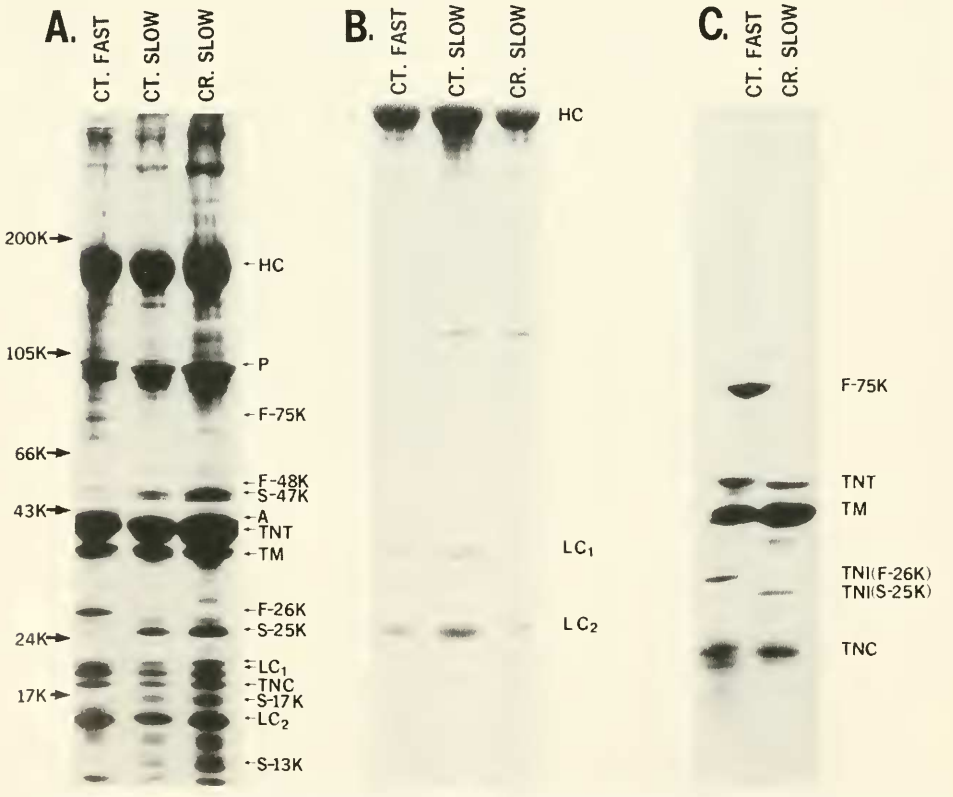

FIGURE 5. Electrophoretic protein patterns of fast and slow muscle from the cutter (CT) and crusher (CR) claw closer muscle of an adult lobster. A, Whole myofibrillar homogenate showing the common proteins such as myosin heavy chain (HC), two myosin light chains (LC1, LC2), actin (A), paramyosin (P), tropomyosin (TM), troponin-C (TNC), and troponin-T (TNT). Proteins unique to fast (F) and slow (S) muscle are so indicated at their respective molecular weights. B, Myosin extract showing heavy and light chains. C, Troponin-tropomyosin extract showing several unique proteins and troponin-I (TNI) (from Costello and Govind, 1984).

themselves. There were three such bands in the electrophoretic pattern for fast muscle seen at F-75K, F-48K and F-26K in Figure 5 and four unique bands for slow muscle at S-47K (doublet form), S-25K, S17K, and S-13K. One of these unique proteins in each fiber type, F-26K in fast muscle and S-25K in slow muscle corresponds to the regulatory protein troponin-I (Fig. 5) Other regulatory proteins include troponin-T (TNT) normally masked by actin and troponin-C (TNC) and tropomyosin (TM) which are common to both fast and slow muscle (Fig. 5).

The earliest stage during development of the paired closer muscles examined electrophoretically was the 4th juvenile stage when the muscles are symmetric in fiber composition. At this stage the closer has almost all of the major proteins common to both adult fast and slow muscle viz. myosin heavy and light chains, actin, paramyosin, and tropomyosin (Fig. 6). A high molecular weight protein at 290K which is common to both types of adult muscle is lacking in the 4th stage muscle. More significantly, however, is the lack of all proteins unique to fast muscle (F-75K, F-48K, and F-26K) and of one protein unique to slow muscle (S-13K) in this juvenile muscle. Furthermore the slow muscle protein S-47K is present in a singlet form in the 4th stage muscle and not in the doublet form characteristic of the adult muscle. These missing proteins are present in the 10th stage muscle, except for S-13K which is still absent in the cutter slow muscle.

The major proteins common to both fast and slow muscle are present in the first juvenile form (4th stage) as are also three of the four proteins unique to slow muscle.

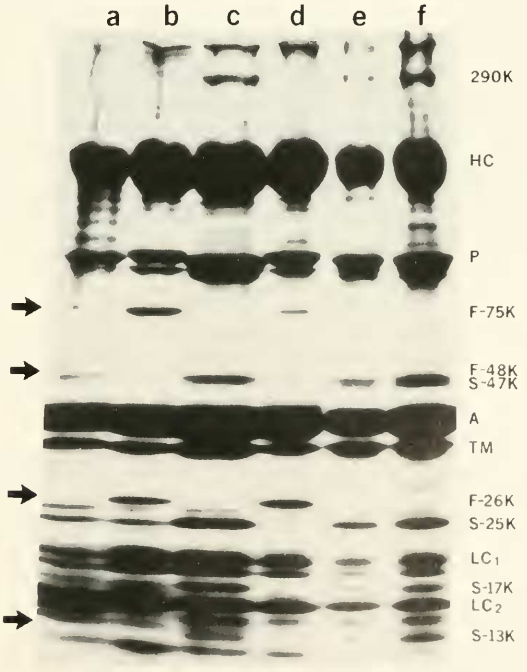

FIGURE 6. Differentiation of the electrophoretic protein pattern of the claw closer muscles. Lane a: undifferentiated muscle of juvenile 4th stage. Lanes b, c: differentiated cutter (fast and slow fibers) and crusher (slow fibers) muscles respectively of juvenile 10th stage. Lanes d, e, f: fully differentiated cutter fast, cutter slow, and crusher slow muscles respectively of an adult. Abbreviations as in Figure 5 (from Costello and Govind, 1984).

During juvenile development the missing unique proteins of fast and slow muscle are expressed. Since some of these unique fast proteins (F-26K, and F-75K) are tentatively identified as troponin I and troponin-tropomyosin complex respectively, their belated appearance suggests a gradual maturation of the regulatory mechanism governing contraction of fast muscle. Moreover, the appearance of these unique proteins during juvenile development not only signals the activation of new genes but underscores the fact that the muscle fibers are differentiating into their adult character.

These biochemical studies do not address the question of how and when the paired closer muscles become asymmetric. In order to answer these questions, individual fibers or at most a small group of fibers taken from areas of the closer muscle known to be either fast or slow according to structural and histochemical tests would have to be analyzed for their protein composition in the first year of development *i.e.,* from the 4th to the 13th stage. This will reveal the protein composition of fibers which are transforming from fast to slow and vice versa.

Contractile properties. The contractile behavior of individual fibers in the adult cutter and crusher muscles has revealed a wide spectrum which has been conveniently grouped into fast, slow, and intermediate types (Jahromi and Atwood, 1971; Costello and Govind, 1983a). Thus fast-follower fibers have a rapid rise to peak tension which is maintained at a plateau and a rapid decay (Fig. 7). Slow-follower fibers show a gradual and continual increase in tension with a decay phase that is equally slow. The intermediate fibers showed a mixture of the tension properties of fast and slow fibers by having an initial rapid rise time followed by a slower rise time. The wide range of contractile behavior encompassed by these three arbitrary categories is seen in the rise time of fibers which extends between 50 to 800 ms for both adult muscles. In larval and early juvenile lobsters the rise time of fibers was between 50 to 400 ms. The slower rise times characteristic of the adult fibers is not present in the 4th juvenile stage and must be acquired during subsequent juvenile development. Indeed there are few slow-follower type fibers in the 2nd, 3rd, and 4th stage muscle, the majority being intermediate and fast-follower types. In the differentiation to asymmetric muscles the slow fiber population increases at the expense of the intermediate fibers in the cutter claw and of the fast fibers in the crusher claw judging by the distribution of these three fiber types during development (Table I).

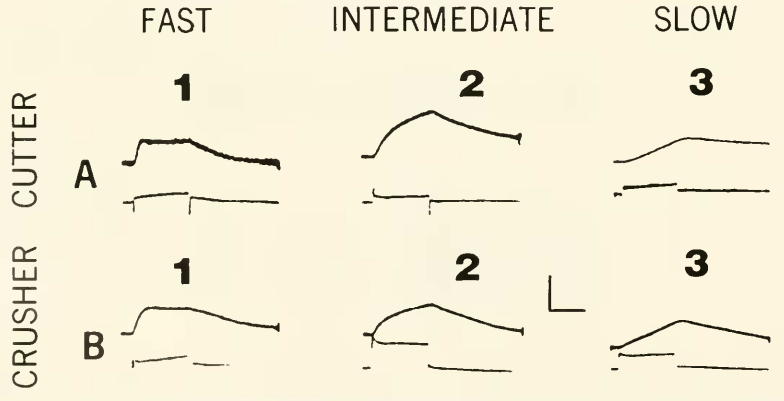

FIGURE 7. Contractile responses of single muscle fibers (upper trace) to short, 800 ms, depolarizing pulses (lower trace) in cutter and crusher claw closer muscles showing representative fast, intermediate, and slow types. Calibration: vertical 5 mg; horizontal, 400 ms. (from Costello and Govind, 1983a).

As a result, the adult crusher muscle has more intermediate and slow fibers and less fast fibers than its cutter counterpart. This would account for the fact that in the intact animal both claws display a wide range of movements from brief, rapid twitches to prolonged, slow contractions (Costello *et al.*, 1984). The asymmetry in contractile types between the paired muscles is therefore in the relative proportion of the three types and not in the fiber types themselves. Given this fact it is interesting to correlate the contractile behavior of these fibers with their SL, ATPase activity and innervation in order to obtain a more comprehensive picture of muscle asymmetry.

Such a correlation (Table II) made for groups of fibers, shows broad agreement with the idea that fast-contracting fibers have high ATPase levels, low oxidative capacities, and short SL, while slow-contracting ones have low ATPase levels, high oxidative capacities and long SL. On an individual basis this three-way correlation does not necessarily hold; *e.g.*, in the crusher all fibers have long SL, low ATPase levels yet can contract rapidly. Finally, when the motor innervation of these bundles of fibers is considered with their other properties, the bundles are seen to be functionally specialized, some for fast, brief contractions (such as the cutter dorsal and proximal bundles) and others for slower, more sustained contractions (such as the central distal bundles).

Opener muscle

As an antagonist to the closer muscle, the opener muscle elevates the dactyl in preparation for the closing action. As such it performs a necessary function, considerably limited in scope, which is reflected by the small size of the muscle compared to the closer. Not surprisingly it has received little attention in the adult claws and none whatsoever during development of the claws.

Structural properties. The frequency histogram of SL from the adult muscles (Fig. 8) shows a range between 6–9 μm for the cutter and 9–11 μm for the crusher, with no overlap between them (Govind *et al.*, 1981). Though the SL of fibers in both adult muscles is >6 μm, the mean SL of the cutter muscle at 8 μm is significantly shorter than that of its counterpart muscle at 10 μm (Fig. 8). Clearly the paired muscles are

TABLE II

Correlation of contractile (rise time) histochemical (ATPase and oxidative capacity) and structural (sarcomere length) properties of closer muscle in different regions of the paired cutter and crusher claws (from Costello and Govind, 1983a)

	Rise time (ms)	ATPase activity	Oxidative capacity	Sarcomere length
Cutter muscle				
dorsal	95	high	low	short
proximal	80	mixed	mixed	short
ventral	236	low	high	long
proximal ventral	326	low	high	long
central distal	489	very low	very high	long
Crusher muscle				
dorsal	232	low	high	long
proximal	189	low	high	long
ventral	256	low	high	long
proximal ventral	379	low	high	long
central distal	554	very low	very high	long

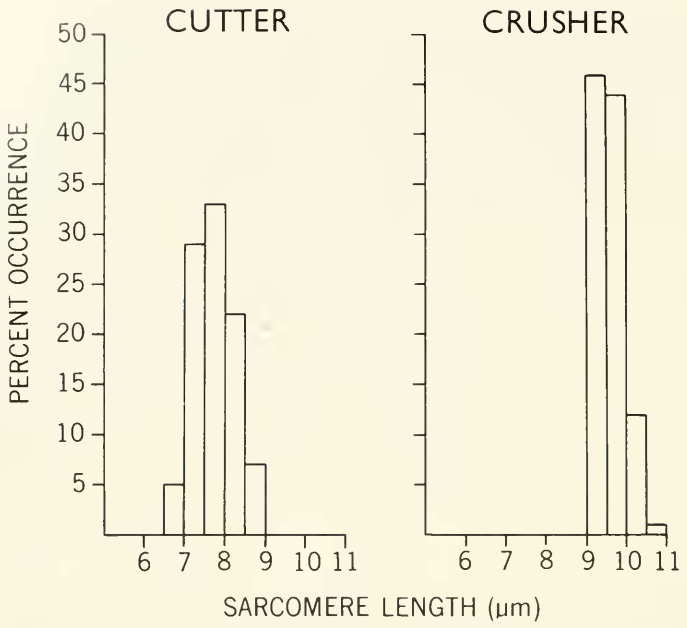

FIGURE 8. Histogram of percent occurrence of muscle fiber types based on sarcomere length in the paired claw opener muscles of an adult lobster. Number of fibers sampled is 158 for each claw (from Govind *et al.*, 1981).

asymmetric in SL though the asymmetry is much more subtle than that seen for the closer muscle.

Histochemical properties. Since cross-sections of the entire claw were taken for histochemistry of the closer muscle (Fig. 3), the opener muscle was always included. The opener muscle showed low specific activity of myofibrillar ATPase typical of slow muscle judging by the light staining compared to the fast fibers of the cutter closer muscle. The staining pattern remains virtually unchanged between the paired claws during development resulting in the symmetry seen in the adult cutter and crusher muscles. Proximal slow fibers of both opener and closer muscles in both claws stain less intensely for ATPase than the remainder (Kent and Govind, 1981), which suggests subdivision within the slow category.

Biochemical properties. The electrophoretic protein pattern of the opener muscle is similar in both adult cutter and crusher claws and resembles the pattern of the slow fibers from the closer muscle (cf. Fig. 5). Thus all the unique proteins of the slow fibers of the closer muscle are found in the opener muscle as well as those represented by the bands at S-47K, S-25K, S-17K, and S-13K. The only difference is the presence of an unidentified protein at 122K which is not found in the closer muscle.

DEVELOPMENT OF NEURONAL ASYMMETRY

The innervation of the limb muscles in crustaceans is well established from the classical work of Wiersma (1961). The claw closer muscle is supplied by three motor axons (two excitors and an inhibitor) which are well-characterized in the adult and whose development has been followed. The claw opener muscle receives only two

axons (an excitor and an inhibitor) which have not been as well studied as the closer axons. In addition there are the large numbers of different sensory receptors landscaping the claw for which little information is available.

Motoneurons to closer muscle

Number and type. The two excitor axons to the adult muscle are differentiated into a fast closer excitor (FCE) and a slow closer excitor (SCE) on the basis of the contractions they evoke (Wiersma, 1955). Though these contractions are qualitatively similar between the paired claws, those to the cutter are more rapid and fatigue more readily than those to the crusher (Govind and Lang, 1974, 1979). Thus the homologous motoneurons are asymmetric in the adult.

Excitatory innervation of the closer muscle is present at the time of hatching with well-defined neuromuscular terminals containing synaptic vesicles and presynaptic dense bars (Fig. 9) denoting active sites of transmitter release at synapses (King and Govind, 1980). The number and type of excitatory axons is however not known in these 1st larval stages. Two excitor axons are physiologically identifiable in the 2nd larval stage with one of them being reminiscent of the FCE (Hill and Govind, 1984). By the 3rd larval stage, the two axons are sufficiently well-differentiated to be recognizable as putative FCE and SCE axons and their physiological identity is firmly established in the succeeding juvenile stages (Costello *et al.*, 1981), though exactly when the homologous motoneurons diverge into cutter and crusher types is not known. Morphologically the motoneurons mature within the first year of development so that by the 10th juvenile stage they resemble their adult counterparts (Fig. 10) (Hill and Govind, 1983). The general form for both FCE and SCE neurons is similar, consisting of an antero-ventrally located soma from which a single neurite rises vertically to the dorsal surface of the ganglion. The neurite courses diagonally across the ganglion to the second root which it enters as an axon. Dendritic branches which

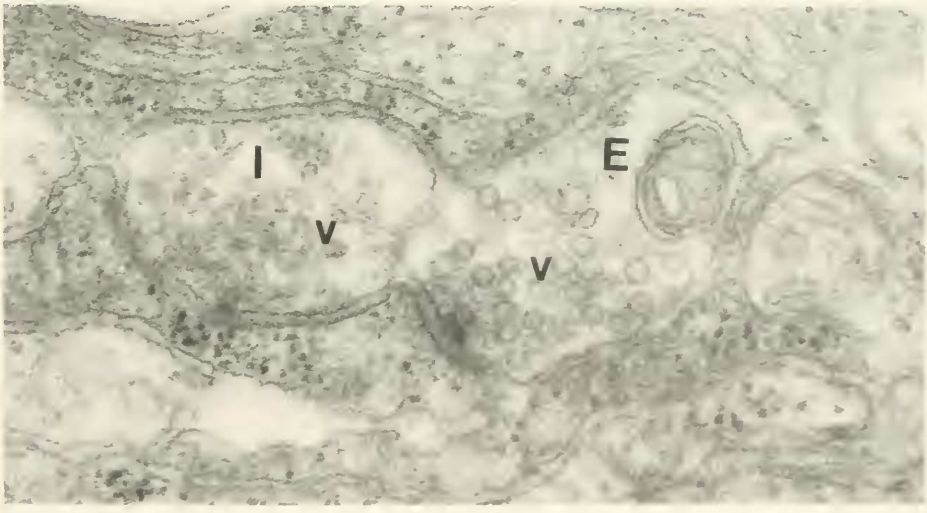

FIGURE 9. Excitatory neuromuscular terminal (E) recognized by spherical synaptic vesicles (v) adjacent to an inhibitory terminal (I) which contains irregularly-shaped vesicles in the juvenile 4th stage claw closer muscle. These two types of terminals also occur in the larval 1st stage and adult muscle. Magnification 20,000×. (from King and Govind, 1980).

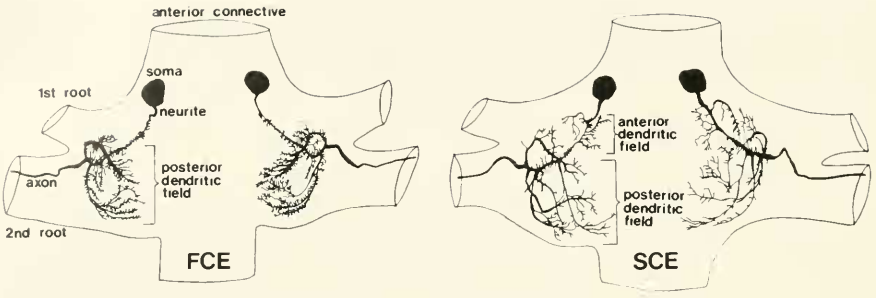

FIGURE 10. Camera lucida drawings of cobalt-filled motoneurons of paired FCE and SCE motoneurons in juvenile lobsters with cutter (right side) and crusher (left side) claws. Magnification, 40×. (from Hill and Govind, 1983).

arise from the neurite and are restricted to their respective hemiganglia, differ between FCE and SCE neurons. The SCE has a much more elaborate dendritic field than the FCE. Thus, whereas the FCE has only a distal dendritic field of two primary branches, the SCE has a distal field of several primary branches and a proximal field as well. In view of the striking asymmetry in behavior, external form and muscle composition of the paired claws, there was surprisingly no asymmetry between the homologous motoneurons.

A closer inhibitor (CI) axon is present in the 1st larval stage muscle (Fig. 9) (King and Govind, 1980) judging from the occurrence of neuromuscular terminals populated by ellipsoid-shaped synaptic vesicles which are characteristic of inhibitory terminals (Atwood *et al.,* 1972). Whether there is a single CI cannot be deduced from this type of morphological evidence. In the juvenile 4th stage, however, a single class of inhibitory junctional potentials (ijp) is seen with stimulation of the closer nerve suggesting the presence of a single CI (Costello *et al.,* 1981). The adult closer muscles receive a lone CI (Hill and Govind, 1981) which is seen to be shared with several other cheliped muscles (Hill and Lang, 1979).

Distribution. In the juvenile 4th, 5th, and 6th stages, the innervation pattern of the FCE and SCE axons is similar between the paired muscles (Table III) (Lang *et al.,* 1980; Costello *et al.,* 1981). The majority of fibers receive both axons while a small number receive each axon exclusively. In the adult the pattern is dramatically different between cutter and crusher muscles. Most of the fibers in the cutter receive

TABLE III

Distribution of innervation by FCE and SCE axons in claw closer muscle of juvenile lobsters where the pattern is similar between the paired claws and in adult lobsters where the pattern differs between the paired (cutter and crusher) claws (from Costello et al., *1981).*

	% innervation		
	FCE	SCE	FCE + SCE
Stage 4	9	5	86
Stage 5	18	5	77
Stage 6	9	9	82
Adult cutter	64	16	20
Adult crusher	15	18	67

FCE only, a few receive either the SCE only or both SCE and FCE together. In contrast, most of the crusher fibers receive both axons, while a few receive either FCE or SCE exclusively. This signifies a clear change in the innervation patterns between juvenile and adult muscles. Since the paired juvenile muscles are symmetrical in their innervation they may be regarded as being undifferentiated compared to the adult cutter and crusher muscles which have their own peculiar innervation pattern representing the differentiated condition. From the undifferentiated juvenile state where the large majority (80%) of fibers received both FCE and SCE axons, selective elimination of SCE would result in the adult distribution of 64% FCE innervation in the cutter. Synaptic elimination, on a smaller scale, of the FCE elements would give rise to the adult value of 16% SCE innervation. Similar processes would operate in finalizing the innervation to the crusher muscle where synapse elimination would affect fewer fibers since only a small number are supplied exclusively by each axon. According to this scheme the final pattern of innervation is refined by selective elimination of cutter FCE or SCE synapses from an initial (juvenile) condition where both axons are present. There may well be alternative methods for achieving the adult innervation patterns, such as the generation of new synapses, though the proposed mechanism is the most parsimonious one.

The distribution of the CI axon has not been mapped out for either developing (juvenile) or adult lobsters. In the few instances where CI has been detected, it was found on fibers with SCE innervation (Costello *et al.*, 1981; Hill and Govind, 1982).

Synaptic properties. In adult lobsters the neuromuscular synapses provided by the FCE and SCE axons differ in their physiological and fine structural properties. Thus the amplitude of the excitatory junctional potential (ejp) at 1 Hz stimulation is generally larger for the FCE than for the SCE synapses (Fig. 11) (Govind and Lang, 1974; Costello *et al.*, 1981). Conversely the degree of facilitation of the ejps calculated as the ratio of the ejp amplitude at 10 and 1 Hz is greater for the SCE than the FCE synapses. The SCE synapses were more fatigue-resistant and showed better recovery following fatigue than their FCE counterparts. FCE fine structure is relatively simple in having small-diameter terminals each with few synaptic vesicles, a single long synapse and little if any postsynaptic apparatus (Hill and Govind, 1981). The SCE innervation is more complex in having a wide size range of terminals each with many synaptic vesicles, several short synapses, and an extensive postsynaptic apparatus. The above data shows a clear distinction between neuromuscular synapses of the FCE and SCE axons.

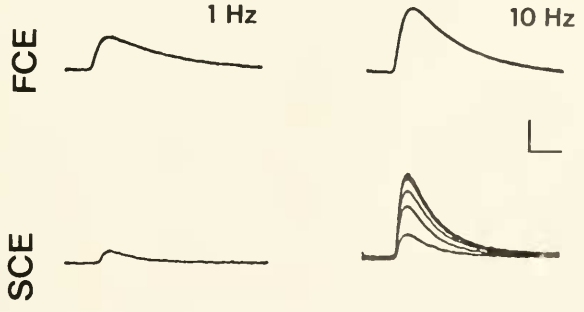

FIGURE 11. Synaptic properties represented by the amplitude of the ejp at 1 Hz stimulation and its degree of facilitation at 10 Hz for the FCE and SCE axons in an adult cutter closer muscles. Vertical calibration, FCE, 5 mV; SCE 4 mV. Horizontal calibration, 20 ms. (Costello *et al.*, 1981).

The physiological data reveals no differences in homologous synapses between cutter and crusher claws except perhaps that the FCE synapses show a greater maximal EJP amplitude in the cutter compared to the crusher claw. The other difference between the paired claws is that synapses of both axons tended to be more fatigue-resistant in the crusher compared to the cutter claw (Govind and Lang, 1974).

The development of neuromuscular synapses from the excitatory axons has been studied using electrophysiology and electron microscopy. In a few recordings made from the larval 2nd stage muscle, large ejps of approximately 10 and 20 mV were characteristic of putative SCE and FCE synapses respectively (Hill and Govind, 1984). These large synaptic potentials of the FCE axon often produced secondary regenerative responses. In a larger sampling of synapses from the larval 3rd stage the mean ejp size was 6 mV with a range of 4 to 10 mV for the FCE synapses. The SCE synapses were considerably smaller with a mean of 3 mV and a range of 2 to 4 mV. The amount of facilitation was similar for the two types of synapses. However, the FCE synapses often produced regenerative responses and displayed fewer transmission failures than the SCE synapses. In the juvenile 4th, 5th, and 6th stages (Costello *et al.*, 1981) ejps of the FCE displayed a wide range in amplitude though the mean size was similar to the larval and adult forms signifying that they had reached their final condition. On the other hand, ejps of the SCE axon in these juvenile stages had as narrow a range of amplitudes as those in the larval stage. The wider spread in ejp amplitude typical of the adult SCE synapses must presumably come with maturation. The other difference between FCE and SCE synapses in the juvenile lobsters is the fact that the former synapses are much more fatigue-resistant than the latter; a situation which is exactly the reverse of that found in adult lobsters.

In terms of the size of the ejp, synapses differentiate into FCE and SCE types early in the larval stages while the properties of facilitation and fatigue-sensitivity mature later during juvenile development.

Structural aspects of the development of excitatory synapses were examined by serial section electron microscopy of the closer muscle in a larval 1st stage, a juvenile 4th stage, and an adult lobster (King and Govind, 1980). No attempt was made to determine whether the terminals belonged to the FCE or SCE axons. There was a tremendous proliferation of excitatory innervation from the 1st larval stage where it was restricted to four discrete locations over the entire muscle to individual muscle fibers in the adult. Concomitantly there is a ten-fold and significant increase in the mean size of synapses between larval and adult stages (Table IV). The mean size of terminals varied considerably among the three stages examined and showed no consistent trend. On the other hand, the presynaptic dense bars, representing active sites of transmitter release were consistently similar in size and were found in the majority (>60%) of synapses. Synaptic development therefore consists of an increase in number and size of excitatory synapses which occur in tandem with the increase in mass of the closer muscle. From within this overall pattern of synaptic development, there is a need to distinguish between FCE and SCE synapses in order to understand how their final distribution within the closer muscle forms. A start has been made in this direction by examining physiologically identified FCE and SCE terminals in juvenile lobsters (Hill and Govind, 1981). The FCE innervation is relatively simple, consisting of small terminals each with a single synapse, few synaptic vesicles and limited postsynaptic apparatus. In contrast the SCE innervation is more complex, having larger and more variable terminals each with several short synapses, many synaptic vesicles, and an extensive postsynaptic apparatus.

Firing patterns. The *in vivo* activity of the FCE and SCE axons during reflex closing of the claws was analyzed in one to three-year-old juvenile lobsters with

TABLE IV

Quantitative comparison of excitatory nerve terminals, synapses, and presynaptic dense bars in the claw closer muscle of a larval (1st stage), juvenile (4th stage), and adult lobster (from King and Govind, 1980)

	1st stage	4th stage	Adult
Nerve terminals:			
Length of muscle fiber serially sectioned (μm)	10.23	11.12	12.05
Total number	6	5	5
Mean surface area (μm^2)	34.63	53.73	19.49
($\bar{x} \pm$ S.E.M.)	±10.93	±32.93	±10.55
Synapses:			
Number completely sectioned	29	51	15
Mean number per terminal	4.83	10.20	3.20
($\bar{x} \pm$ S.E.M.)	±1.79	±5.04	±0.89
Mean surface area (μm^2)	0.079	0.136	0.805
($\bar{x} \pm$ S.E.M.)	±0.010	±0.012	±0.174
Presynaptic dense bars:			
Total number	22	40	13
Mean surface area (μm^2)	0.018	0.016	0.017
($\bar{x} \pm$ S.E.M.)	±0.005	±0.002	±0.002
Mean number per synapse	0.759	0.784	0.867
($\bar{x} \pm$ S.E.M.)	±0.128	±0.081	±0.236

dimorphic claws (Costello *et al.*, 1984). While the dactyl was free to move, the rest of the claw and the animal was immobilized in order to permit recordings of ejps from the closer muscle fibers. Under these conditions, the FCE fired only during rapid closing at a lower frequency and duration than the SCE which fired only during slow closing of the cutter claw (Table V). However, the crusher FCE and SCE axons were active during fast and slow closing respectively and their firing patterns were similar. This similarity was also found between the two axons during maintained closing of the crusher. Thus a clear distinction is found between FCE and SCE axons in the cutter but not in the crusher claw.

When the homologous motoneurons are compared, the FCE of the crusher has a significantly higher firing frequency and burst duration than its cutter counterpart during fast closing of the claw (Table V). The homologous SCEs, however, displayed

TABLE V

In vivo *firing frequency and burst duration of FCE and SCE axons during fast, slow, and maintained closing of cutter and crusher claws of intact lobsters (from Costello* et al., *1984)*

Claw type	Closing behavior	Motoneuron type	Frequency (Hz) $\bar{x} \pm$ S.D.	Burst duration (ms) $\bar{x} \pm$ S.D.	n
Cutter	fast	FCE	2 ± 2	55 ± 26	46
Cutter	slow	SCE	37 ± 24	361 ± 143	51
Cutter	maintained	SCE	15 ± 9	1963 ± 1351	31
Crusher	fast	FCE	37 ± 27	215 ± 94	44
Crusher	maintained	FCE	10 ± 9	1010 ± 630	13
Crusher	slow	SCE	31 ± 12	406 ± 175	17
Crusher	maintained	SCE	18 ± 13	1278 ± 707	23

a close similarity in their firing patterns during slow closing. Maintaining the closed claw was achieved by the FCE and SCE axons in the crusher but only by the SCE in the cutter claw though all three axons were similar in their firing patterns. Thus the FCE alone showed an asymmetry in firing patterns between cutter and crusher claws in intact juvenile lobsters.

In contrast to the above, the *in vitro* activity of the adult motoneurons shows a clear asymmetry between FCE and SCE in *both* claws and between *both* homologs (Govind and Lang, 1981). Activity of the motoneurons was recorded from their respective somata in response to electrical stimulation of the mixed nerve roots in an isolated claw-ganglion preparation. In both claws, the FCE fired at a lower frequency and for a shorter time than the SCE. When homologous somata were examined, the crusher FCE and SCE produced higher frequencies and longer bursts of spikes than their cutter counterparts (Fig. 12). Since this asymmetry was found in response to both sensory stimulation via the 2nd nerve root and depolarization of the soma it could have both an extrinsic (sensory) and intrinsic (built-in) origin.

The motoneurons also produce a distinct pattern of paired impulses (Costello *et al.*, 1981; Govind and Hill, 1982) which are functionally more effective in generating muscle tension than uniformly spaced impulses of the same average frequency (Ripley and Wiersma, 1953; Govind and Lang, 1974). In intact juvenile lobsters, paired impulses with interpulse intervals of between 8 to 13 ms, were found for both FCE and SCE axons in both claws. The only indication of asymmetry in this firing pattern

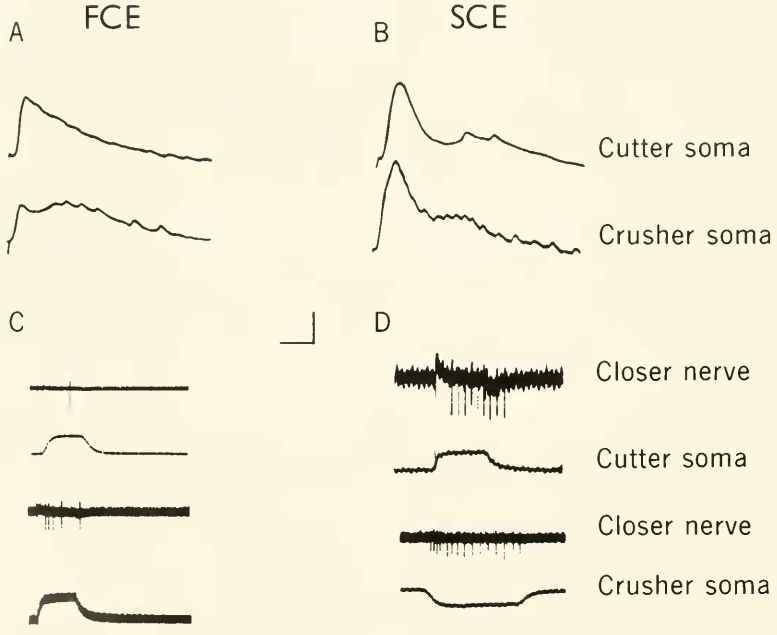

FIGURE 12. Firing patterns of homologous FCE and SCE neurons recorded either from their somata (A, B) in response to sensory stimulation via the 2nd nerve root or from the closer nerve (C, D) in response to depolarization of their somata. For each homologous pair, the crusher motoneuron shows a greater response than its cutter counterpart. Vertical calibration, 4 mV in A; 10 mV in B; 1 μA in C lower trace; 2 μA in C upper trace and D. Horizontal calibration, 40 ms in A, B, D lower trace; 100 ms in C, D upper trace (from Govind and Lang, 1981).

was for the homologous FCE axon which produced paired impulses almost all the time in the crusher but only 25% of the time in the cutter. The homologous SCE axons resembled each other in producing paired impulses 60% of the time. In isolated claw-ganglion preparations of adult lobsters, depolarization of the soma gave rise to paired impulses in FCE and SCE motoneurons thereby strongly implicating an endogenous mechanism for the generation of this patterned activity.

The development of the characteristic firing patterns for the FCE motoneurons alone has been examined in intact juveniles (Costello and Govind, 1983b; unpub.) where closing has been reflexly evoked. In the juvenile 4th and 5th stage the homologous FCE fire at a similar frequency of 100–150 Hz for 150–200 ms. In the subsequent juvenile stages till about the 12th stage, both the frequency and duration of firing decreases dramatically in the putative cutter claw to <10 Hz for <50 ms which approximates the adult condition. In the putative crusher the duration fluctuates between 100–200 ms while the frequency gradually decreases to <50 Hz which is reminiscent of the adult condition. The activity patterns of the homologous FCE have essentially matured by the time the lobster is a year old.

The closer muscles have a timetable similar to that of the FCEs in achieving their final composition of fiber types. Whether there is any causal relationship between the development of asymmetry in the firing patterns of the homologous FCE motoneurons and in the fiber composition of the closer muscles cannot be deduced from this correlation. However, transformation of fast fibers to slow and the resultant differentiation of a crusher muscle with all slow fibers can be prevented by denervation or tenotomy in the early juvenile stages (Govind, 1981; Govind and Kent, 1982). Since these treatments reduce or eliminate active muscle tension mediated by motor impulses, they implicate the motoneurons in directing the differentiation of muscle fiber types. On the other hand, these experiments also suggest that it may be the overall level of active muscle tension which transforms fast fibers to slow. Consequently, the trigger for muscle transformation may well reside in the level of motoneuronal activity of both excitors, FCE and SCE, and the inhibitor, CI, axons. The claw muscle experiencing the higher level of motor activity would become the crusher while its counterpart muscle would become the cutter.

Motoneurons to the opener muscle

The innervation to this muscle in lobsters has received scant attention compared to the very extensive studies of the homologous muscle in crayfish (reviewed by Atwood, 1976). The discovery of subtle asymmetries in the neuromuscular system in the opener muscle between cutter and crusher claws (Govind *et al.*, 1981) has initiated a more detailed current investigation (G. Kass-Simon and K. Mearow, unpub.) which provides the basis for most of the comments given here unless otherwise acknowledged.

Number and type. The opener muscle in the limbs of decapod crustaceans is supplied by an excitor (OE) and inhibitor (OI) motoneuron (Wiersma, 1961). While the OE also innervates the stretcher muscle in the next proximal segment, the OI is a private motoneuron. However, more recent evidence suggests that the CI also innervates the opener muscle (T. J. Wiens, pers. comm.). The OE is reminiscent of a slow excitor axon as it does not cause rapid opening of the dactyl and is more fatigue-resistant. The development of excitatory and inhibitory innervation has not been examined in the lobster though both types of synapses are present immediately after hatching in the homologous opener muscle in the crayfish (Atwood and Kwan, 1976).

Distribution. Being the only excitor axon, OE may be expected to innervate all fibers of the adult opener muscle. The presence of OI and CI however has not been detected in all fibers examined suggesting a regional distribution of innervation for each of these two axons which may account for the fact that they were not recognized as separate axons in the past.

Synaptic properties. Generally the amplitude of the ejps are small ranging from <1 mV to 5 mV; many being visible only after a bout of high frequency stimulation designed to produce facilitation and summation. All of the excitatory synapses showed moderate to strong facilitation with repeated stimulation. The OI synapses also gave small junctional potentials which were either hyperpolarizing or depolarizing in sign. These ejps exerted considerable postsynaptic inhibition judging from the fact that they reduced the size of the ejp considerably. Almost complete elimination of the ejp occurred occasionally suggesting pre-synaptic inhibition similar to that found in the homologous motoneurons in the crayfish opener muscle. In terms of their physiology the OE and OI synapses in lobster resemble their counterparts in crayfish (Atwood and Bittner, 1971). Consequently they may also resemble them in ultrastructure which has been extensively described in crayfish (Jahromi and Atwood, 1974).

Firing patterns. The crusher OE has a higher frequency of firing and longer burst duration than its cutter counterpart in response to nerve root stimulation in isolated ganglia of adult lobsters (Govind *et al.,* 1981). The crusher OE is also more resistant to fatigue when stimulated repetitively than the cutter OE. This asymmetry in firing patterns between homologous OE motoneurons *in vitro* forebodes a similar asymmetry in the intact lobster. The ontogeny of these firing patterns is unknown.

Sensory neurons

In a singular attempt to document asymmetry in the sensory system between paired cutter and crusher claws, the number and size of axons was determined in the nerve roots to a juvenile lobster (Govind and Pearce, 1984). The nerve roots are mixed, containing both sensory and motor axons. However, since the motor axons are bilaterally constant and relatively few in number, the majority of axons in the nerve roots are sensory. The total numbers of axons in the first root were approximately 16,000 for the crusher and 13,000 for the cutter; which gave a crusher-cutter ratio of 1.22. For the second root the counts were 119,000 for the crusher and 124,000 for the cutter which gave a ratio of 0.96. The slight asymmetries in the roots proved not to be significant in random samples from homologous regions. Furthermore, a representative sampling of the axon diameters showed a parallel distribution in all size classes between crusher and cutter claws. Consequently, there does not appear to be a asymmetry in the numbers and sizes of sensory axons between the paired claws in a juvenile lobster. However, in adults the external dimorphism between the paired claws is much more pronounced than in the juvenile and there is the possibility that the sensory system may be asymmetric.

Similarly, no differences in the distribution of four different types of cuticular hair organs were detected between cutter and crusher claws of "subadult" lobsters (Solon and Cobb, 1980). These four cuticular hair organs which are regarded as mechanosensory in function differ basically in the length of the sensilla: type I are the longest (70–130 μm), type II slightly smaller at 30–60 μm, type III still smaller but located in a raised protuberance, and type IV are simply 1 μm long conical hairs occurring in clusters. Types II and III differed in distribution between dorsal and ventral sides and among different areas of the claw. More interesting was their dis-

tribution in a juvenile lobster with symmetrical claws. Type IV receptors were just as ubiquitous in the juvenile as in the subadult. Type III receptors, however, had a lower density in the juvenile than in the subadult denoting the addition of these hair organs during growth of the claws. On the other hand, types I and II with a higher density in the juvenile than in the subadults are apparently not added during growth. These differences in density of particular types of hair organs between juveniles and subadults may reflect changes in behavior during development which have been documented previously (Lang *et al.*, 1977c).

Comparison with other Asymmetric Systems

Claw asymmetry is not uncommon among crustaceans and it may be instructive to review how it arises during development in fiddler crabs and how it is maintained during regeneration in snapping shrimps. Adult male fiddler crabs have a hypertrophied major claw used for courtship and defence and a minor claw used for feeding and grooming (reviewed by Crane, 1977). The asymmetry in external form is matched by an asymmetry in muscle mass (Rhodes, 1977), soma size, and dendritic field of the motoneurons (Young and Govind, 1983), and in the numbers of sensory axons (Govind and Pearce, 1984). Early in development the paired claws are symmetrical. The asymmetry develops following the loss of one of the paired claws during a critical period which extends from the megalopa to a young crab stage (Morgan, 1923, 1924; Yamaguchi, 1977). If both claws are removed at this stage then no major claw develops; if both are kept intact during this critical period, then paired major claws develop. Consequently the loss of a claw during development triggers the remaining one to differentiate into a major claw in male fiddler crabs. Once the claw asymmetry is established it remains fixed and removal of either major or minor claw will cause the same type to regenerate. This is similar to the situation in lobsters but unlike that in snapping shrimps where claw laterality is not fixed in the adult.

The major or snapper claw in Alpheid shrimps is used in defence when it ejects a jet of water on closing and at the same time makes a loud popping sound; the minor or pincer claw is used for feeding and grooming. In adult shrimps, autotomy of the snapper results in the regeneration of a pincer in its place while the existing pincer transforms into a snapper (Prizbram, 1901; Wilson, 1903). This pincer to snapper transformation involves several changes: a hypertrophy and differentiation in the external form, a hypertrophy of the motoneuron somata to the closer muscle (Mellon *et al.*, 1981), a hypertrophy of the closer muscle and the transformation of its fast fibers to slow, and an increase in facilitation of the excitatory neuromuscular synapses (Stephens and Mellon, 1979). Transformation is either prevented if the nerve to the pincer is transected at the time of snapper removal (Wilson, 1903) or promoted if the nerve to the snapper alone is transected (Mellon and Stephens, 1978). The pincer can be regarded as an undifferentiated snapper which is arrested in its development by the existing contralateral snapper. Once this inhibition is removed by autotomy of the snapper or transection of its nerve, the pincer completes its differentiation to a snapper and at the same time arrests the development of the newly regenerating claw to a pincer type. Clearly, the maintenance of claw asymmetry and its reversal in adult snapping shrimps is under neural control (Mellon, 1981). This is similar to how claw type is determined in juvenile lobsters (Govind, 1981) where denervation of one claw causes the contralateral one to become the crusher (Govind and Kent, 1982). However, in lobsters, once claw asymmetry is determined during juvenile development, it remains fixed throughout adult life whereas in snapping shrimps it can be altered in the adult.

Future Prospects

One aspect of our fascination with asymmetric systems, whether it be cerebral dominance in humans or claw lateralization in lobsters, lies in being able to understand how it arises from a bilaterally symmetrical body plan. Such a goal is feasible in the neuromuscular system of the lobster claw because certain features of this system are of advantage in studying development. First the lobster has a protracted period of development, consisting of a 9–11 month embryonic period, a two-week larval period, and a 5–7 year juvenile period (Herrick, 1895, Hughes *et al.,* 1972) which is divided into discrete stages by the molt cycle. All these stages can be reared in the laboratory (Hughes *et al.,* 1974; Lang, 1975). Second, there are only two muscles, the antagonistic opener and closer, which make up the claw. Third, each muscle is innervated by few motoneurons: two excitors and an inhibitor in the closer and a single excitor and two inhibitors in the opener (Wiersma, 1961; T. J. Wiens, pers. comm.). Fourth, and perhaps most important of all, is the fact that claw laterality is determined during a critical two-week period of juvenile development, between the 4th and 5th stages, when the claws can be experimentally manipulated (Emmel, 1908; Lang *et al.,* 1978).

Indeed, manipulations such as tenotomy of the opener or closer muscle, or denervation can suppress the differentiation of a crusher claw, resulting in lobsters with paired cutter claws (Govind and Kent, 1982). In terms of the fiber composition of the closer muscle, this means that fast fibers are prevented from transforming to slow because of a lack of nerve-mediated muscle tension. Since there are only three motoneurons to the closer muscle, each uniquely identifiable, it is possible to examine the influence of each on muscle development. Experimental manipulations of these motoneurons such as selective deletion or electrical stimulation during the critical juvenile period, should pinpoint the role of motoneurons in the differentiation of muscle fiber types.

The experiments proposed above would test the hypothesis that it is the difference in motor output in the paired claws that determines laterality. The claw receiving the greater overall motor output during the critical developmental period transforms its fast fibers to slow and becomes the crusher muscle with all slow fibers. In the absence of a certain level of motor output this transformation is prevented and the closer muscle remains with predominantly fast fibers (Lang *et al.,* 1978) characteristic of the cutter muscle which is presumably the primitive condition. As a corollary, by controlling the motor output to the juvenile undifferentiated muscle we should be able to produce a crusher not only on a prescribed side but on both sides. These experiments, currently in progress, would explain how the asymmetry in muscle fiber composition arises during development.

There is still a need to explain the asymmetry in firing patterns of the homologous motoneurons, specifically, to what extent are they due to the intrinsic (cable) properties of the motoneurons or to extrinsic (synaptic) influences. This will necessitate examining the electrical properties of the homologous motoneurons in the juvenile stages and their synaptic input. If the asymmetry in firing patterns is influenced by the synaptic input we would need to explore its nature and number. This involves primarily the sensory system of the claws though ascending and descending inputs within the ganglion can also influence the motoneuron firing patterns.

Apart from the above experiments revolving around the sensory system and ganglion there is the need to explain the differences in the distribution of the homologous motoneurons onto the closer muscles. From the juvenile condition where the majority of fibers in both muscles are innervated by both excitatory axons, the cutter closer muscle has predominantly FCE innervation while the crusher muscle has predomi-

nantly mixed, FCE and SCE, innervation (Costello *et al.*, 1981). Can this asymmetry in the pattern of synaptic connections be explained by the selective elimination of synapses in the developing cutter muscle, as has been seen in a lobster abdominal muscle (Stephens and Govind, 1981). An equally challenging task would be to understand why such an asymmetry arises: is it due to competition between the motoneurons or is it influenced by the muscle fiber properties?

Finally, a significant component missing from the present consideration of the claw neuromuscular system is the inhibitory (CI) motoneuron. There is a clear need to examine both its central and peripheral mechanisms in order to establish its role in claw asymmetry and to follow its development.

Acknowledgments

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